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TITLE OF THE INVENTION (280 characters)

COMPOSITIONS AND METHODS FOR REGULATING NK CELL ACTIVITY

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U.S. PATENT APPLICATION

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Invention: COMPOSITIONS AND METHODS FOR REGULATING NK CELL ACTIVITY

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SPECIFICATION

753276

COMPOSITIONS AND METHODS FOR REGULATING NK CELL ACTIVITY

Field of Invention

The present invention relates to novel compositions and methods for regulating an immune response in a subject. More particularly, the invention relates to specific antibodies that regulate the activity of NK cells and allow a potentiation of NK cell cytotoxicity in mammalian subjects. The invention also relates to fragments and derivatives of such antibodies, as well as pharmaceutical compositions comprising the same and their uses, particularly in therapy, to increase NK cell activity or cytotoxicity in subjects.

Background

Natural killer (NK) cells are a sub-population of lymphocytes, involved in non-conventional immunity. NK cells can be obtained by various techniques known in the art, such as from blood samples, cytapheresis, collections, etc.

Characteristics and biological properties of NK cells include the expression of surface antigens including CD16, CD56 and/or CD57, and the absence of the alpha/beta or gamma/delta TCR complex expressed on the cell surface; the ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes; the ability to kill tumor cells or other diseased cells that express a NKR-ligand; the ability to release protein molecules called cytokines that stimulate or inhibit the immune response; and the ability to undergo multiple rounds of cell division and produce daughter cells with similar biologic properties as the parent cell. Within the context of this invention "active" NK cells designate biologically active NK cells, more particularly NK cells having the capacity of lysing target cells. For instance, an "active" NK cell is able to kill cells that express a NKR-ligand and fail to express "self" MHC/HLA antigens (KIR-incompatible cells).

Based on their biological properties, various therapeutic and vaccine strategies have been proposed in the art that rely on a modulation of NK cells. However, NK cell activity is regulated by a complex mechanism, that involves both stimulating and inhibitory signals. Accordingly, effective NK cell-mediated therapy requires both a stimulation of these cells and a neutralization of inhibitory signals.

NK cells are negatively regulated by major histocompatibility complex (MHC) class I-specific inhibitory receptors (Kärre et al., 1986; Öhlén et al, 1989). These specific receptors bind to polymorphic determinants of major histocompatibility complex (MHC) class I molecules or HLA and inhibit natural killer (NK) cell lysis. In humans, a family of receptors termed killer Ig-like receptors (KIRs) recognize groups of HLA class I alleles.

There are several groups of KIR receptors, including KIR2DL, KIR2DS, KIR3DL and KIR3DS. KIR receptors having two Ig domains (KIR2D) identify HLA-C allotypes: KIR2DL2 (formerly designated p58.1) or the closely related gene product KIR2DL3 recognizes an epitope shared by group 2 HLA-C allotypes (Cw1, 3, 7, and 8), whereas KIR2DL1 (p58.2) recognizes an epitope shared by the reciprocal group 1 HLA-C allotypes (Cw2, 4, 5, and 6). The recognition by KIR2DL1 is dictated by the presence of a Lys residue at position 80 of HLA-C alleles. KIR2DL2 and KIR2DL3 recognition is dictated by the presence of a Asn residue at position 80. Importantly the great majority of HLA-C alleles has either an Asn or a Lys residue at position 80. One KIR with three Ig domains, KIR3DL1 (p70), recognizes an epitope shared by HLA-Bw4 alleles. Finally, a homodimer of molecules with three Ig domains KIR3DL2 (p140) recognizes HLA-A3 and -A11.

Although KIRs and other class-I inhibitory receptors (Moretta et al, 1997 ; Valiante et al, 1997a; Lanier, 1998) may be co-expressed by NK cells, in any given individual's NK repertoire, there are cells that express a single KIR and thus, the corresponding NK cells are blocked only by cells expressing a specific class I allele group.

NK cell population or clones that are KIR mismatched, i.e., population of NK cells that express KIR that are not compatible with a HLA molecules of a host, have been shown to be the most likely mediators of the graft anti leukaemia effect seen in allogeneic

transplantation (Ruggeri et al., 2002). One way of reproducing this effect in a given individual would be to use reagents that block the KIR/HLA interaction.

Monoclonal antibodies specific for KIR2DL1 have been shown to block the KIR2DL1 Cw4 (or the like) alleles (Moretta et al., 1993). Monoclonal antibodies against KIR2DL2/3 have also been described that block the KIR2DL2/3 HLACw3 (or the like) alleles (Moretta et al., 1993). However, the use of such reagents in clinical situations would require the development of two therapeutic mAbs, to treat the patients expressing class 1 or class 2 HLA-C alleles, and to use either one or the other or both depending of the patients HLA type.

Also, while cross-reacting antibodies have been reported, they do not exhibit inhibitory activity (Watzl et al., Tissue Antigens 56 (2000) 240). Accordingly, practical and effective approaches in the modulation of NK cell activity have not been made available so far in the art and still require allele-specific intervention using specific reagents.

Summary of the Invention

The present invention now provides novel compositions and methods that overcome the current difficulty in NK cell activation. More particularly, the present invention provides a single type of compositions and methods that facilitate activation of human NK cells in virtually all individuals. The invention indeed discloses novel specific reagents that cross react with various KIR groups and neutralize their inhibitory signals, and can be used in most human subjects to increase NK cell activity.

In a first aspect, the invention provides monoclonal antibodies, as well as fragments and derivatives thereof, wherein said antibody, fragment or derivative cross reacts with several KIR receptors at the surface of NK cells and neutralizes their inhibitory signals. More preferably, the invention discloses a monoclonal antibody that binds a common determinant of human KIR2D receptors and inhibit the corresponding inhibitory pathway. More specifically, the invention discloses a monoclonal antibody that binds KIR2DL1 and KIR2DL2/3 receptors at the surface of human NK cells and inhibits KIR2DL1- and KIR2DL2/3-mediated inhibition of NK cell cytotoxicity. The antibody

specifically inhibits binding of HLA-c molecules to KIR2DL1 and KIR2DL2/3 receptors. More preferably, the antibody facilitates NK cell activity in vivo.

Because KIR2DL1 and KID2DL3 (or KIR2DL2) are sufficient for covering most of the HLA-C allotypes, respectively group 1 HLA-C allotypes and group 2 HLA-C allotypes, the compositions of this invention may be used to effectively activate or potentiate NK cells in most human individuals, typically in about 90% of human individuals or more. Accordingly, a single antibody composition may be used to treat most human subjects, and there is seldom need to determine allelic groups or to use antibody cocktails.

The invention shows, for the first time, that cross-reactive and neutralizing antibodies may be generated, that allow effective activation of NK cells in a broad range of human groups.

A particular object of this invention thus resides in a monoclonal antibody, wherein said antibody binds a common determinant of KIR2D human receptors and inhibits KIR2D-mediated inhibition of NK cell cytotoxicity. The antibody more specifically binds to the same epitope as monoclonal antibody DF200 produced by hybridoma DF200 and/or competes with monoclonal antibody DF200 produced by hybridoma DF200 for binding to a KIR receptor at the surface of a human NK cell.

In a specific embodiment, the monoclonal antibody is monoclonal antibody DF200 produced by hybridoma DF200.

The invention also encompasses fragments and derivatives of such a monoclonal antibody having substantially the same antigen specificity, including, without limitation, a Fab fragment, a Fab'2 fragment, a CDR and a ScFv. Furthermore, the monoclonal antibody may be humanized, human, or chimeric (e.g. a bispecific or functionalised antibody).

The invention also discloses methods of producing such antibodies, comprising:

(a) immunizing a non-human mammal with an immunogen comprising a KIR2D polypeptide;

(b) preparing monoclonal antibodies from said immunized animal, wherein said monoclonal antibodies bind said KIR2D polypeptide,

(c) selecting monoclonal antibodies of (b) that cross react with at least two different serotypes of KIR2D polypeptides, and

(d) selecting monoclonal antibodies of (c) that inhibit KIR2D-mediated inhibition of NK cells.

The invention also relates to pharmaceutical compositions comprising a monoclonal antibody as disclosed above or a fragment or derivative thereof, and a pharmaceutically acceptable carrier or excipient.

The invention also provides methods of regulating human NK cell activity in vitro, ex vivo or in vivo, comprising contacting human NK cells with a monoclonal antibody or a fragment or derivative as defined above. Most preferred methods are directed at increasing the cytotoxic activity of human NK cells, most preferably ex vivo or in vivo, in a subject having a cancer, infectious or immune disease.

Legend to the Figures

Figure 1 : Monoclonal antibody DF200 binds a common determinant of various human KIR2D receptors.

Figure 2 : Monoclonal antibody DF200 inhibits KIR2D-mediated inhibition of KIR2DL1 positive NK cell cytotoxicity (reconstitute lysis) on Cw4 positive target cells .

Detailed description of the Invention

Monoclonal Antibody

The present invention provides novel monoclonal antibodies and fragments or derivatives thereof, that bind common determinants of KIR2D human NK receptors and inhibit their inhibitory signalling pathway. The invention discloses, for the first time, that such cross-reacting and inhibitory antibodies can be produced, which represents an unexpected result and opens an avenue towards novel and effective NK-based therapies, particularly in human subjects.

Within the context of this invention a "common determinant" designates a determinant or epitope that is shared by several members of the human KIR2D receptor group. The determinant or epitope may represent a peptide fragment or a conformational epitope shared by said members. In a specific embodiment, the common determinant comprises an epitope recognized by monoclonal antibody DF200.

Within the context of this invention, the term antibody that "binds" a common determinant designates an antibody that binds said determinant with specificity and/or affinity, e.g., that essentially does not bind with high affinity or with specificity other unrelated motifs or determinant or structures at the surface of human NK cells. More particularly, the binding of a monoclonal antibody according to this invention to said determinant can be discriminated from the binding of said antibody to an other epitope or determinant.

Monoclonal antibodies of this invention are able to inhibit the KIR2D-mediated inhibition of NK cell cytotoxicity. These monoclonal antibodies are thus "neutralizing" or "inhibitory" antibodies, in the sense that they block, at least partially, the inhibitory signalling pathway mediated by KIR2D receptors. More importantly, this inhibitory activity is displayed with respect to several types of KIR2D receptors, so that these antibodies may be used in various subjects with high efficacy. Inhibition of KIR2D-mediated inhibition of NK cell cytotoxicity can be assessed by various assays or tests,

such as binding or cellular assays. In a specific variant, the inhibitory activity is illustrated by the capacity of said antibody to reconstitute lysis of KIR2D positive NK clones on HLA-c positive targets. In an other specific embodiment, the antibody is defined as inhibiting the binding of HLA-c molecules to KIR2DL1 and KIR2DL3 (or the closely related KIR2DL2) receptors, further preferably as its capacity to alter :

- the binding of a HLA-c molecule selected from Cw1, Cw3, Cw7, and Cw8 (or of a HLA-c molecule having an Asn residue at position 80) to KIR2DL2/3; and
- the binding of a HLA-c molecule selected from Cw2, Cw4, Cw5 and Cw6 (or of a HLA-c molecule having a Lys residue at position 80) to KIR2DL1.

In an other variant, the inhibitory activity of a monoclonal antibody of this invention can be assessed in a cell based cytotoxicity assay, as disclosed in the examples.

In an other variant, the inhibitory activity of a monoclonal antibody of this invention can be assessed in a cytokine-release assay.

The monoclonal antibodies of this invention may exhibit partial inhibitory activity, i.e., partially reduce the KIR2D-mediated inhibition of NK cell cytotoxicity. Most preferred antibodies are able to inhibit at least 20%, preferably at least 30%, 40% or 50% or more of the KIR2D-mediated inhibition of NK cell cytotoxicity. Alternatively, preferred antibodies of this invention are able to induce the lysis of matched or HLA compatible or autologous target cell population, i.e., cell population that would not be effectively lysed by NK cells in the absence of said antibody. Accordingly, monoclonals of this invention may also be defined as facilitating NK cell activity in vivo.

In a specific embodiment, the monoclonal antibody binds the same epitope or determinant as monoclonal antibody DF200 (produced by hybridoma DF200) and/or competes with monoclonal antibody DF200 (produced by hybridoma DF200) for binding to a KIR2D receptor at the surface of a human NK cell. In a further specific

embodiment, the monoclonal antibody is monoclonal antibody DF200 (produced by hybridoma DF200).

Monoclonal antibodies of this invention may be produced by a variety of techniques known per se in the art. Typically, they are produced by immunization of a non-human animal with an immunogen comprising a KIR2D polypeptide, and collection of spleen cells (to produce hybridomas by fusion with appropriate cell lines). Methods of producing monoclonal antibodies from various species may be found in Harlow et al (Antibodies: A laboratory Manual, CSH Press, 1988). More specifically, these methods comprise immunizing a non-human animal with the antigen, followed by a recovery of spleen cells which are then fused with immortalized cells, such as myeloma cells. The resulting hybridomas produce the monoclonal antibodies and can be selected by limiting dilutions to isolate individual clones. Antibodies may also be produced by selection of combinatorial libraries of immunoglobulins, as disclosed for instance in Ward et al (Nature 341 (1989) 544).

Preferred antibodies of this invention are prepared by immunization with an immunogen comprising a KIR2D polypeptide, more preferably a human KIR2D polypeptide. The KIR2D polypeptide may comprise the full length sequence of a human KIR2D polypeptide, or a fragment or derivative thereof, typically an immunogenic fragment, i.e., a portion of the polypeptide comprising an epitope, preferably a T or B cell epitope. Such fragments typically contain at least 7 consecutive amino acids of the mature polypeptide sequence, even more preferably at least 10 consecutive amino acids thereof. They are essentially derived from the extra-cellular domain of the receptor.

In a most preferred embodiment, the immunogen comprises a wild-type human KIR2D polypeptide in a lipid membrane, typically at the surface of a cell. In a specific embodiment, the immunogen comprises intact NK cells, particularly intact human NK cells, optionally treated or lysed.

Upon immunization and production of monoclonal antibodies, particular selection steps may be performed to isolate antibodies as claimed. In this regard, in a specific

embodiment, the invention also relates to methods of producing such antibodies, comprising:

(a) immunizing a non-human mammal with an immunogen comprising a KIR2D polypeptide;

(b) preparing monoclonal antibodies from said immunized animal, wherein said monoclonal antibodies bind said KIR2D polypeptide,

(c) selecting monoclonal antibodies of (b) that cross react with at least two different serotypes of KIR2D polypeptides, and

(d) selecting monoclonal antibodies of (c) that inhibit KIR2D-mediated inhibition of NK cells.

The order of steps (c) and (d) can be changed. Optionally, the method may further comprise additional steps of making fragments or derivatives of the monoclonal antibody, as disclosed below. In preferred embodiment, the non-human animal is a mammal, such as a rodent (e.g., mouse, rat, etc.), bovine, porcine, horse, rabbit, goat, sheep, etc. Also, the non-human mammal may be genetically modified or engineered to produce "human" antibodies.

In an other variant, the method comprises:

(a) selecting, from a library or repertoire, a monoclonal antibody or a fragment or derivative thereof that cross reacts with at least two different serotypes of KIR2D polypeptides, and

(b) selecting an antibody of (a) that inhibits KIR2D-mediated inhibition of NK cells.

The repertoire may be any (recombinant) repertoire of antibodies or fragments thereof, optionally displayed by any suitable structure (e.g., phage, bacteria, synthetic complex, etc.). Selection of inhibitory antibodies may be performed as disclosed above and further illustrated in the examples.

Fragments and Derivatives of a Monoclonal Antibody

Fragments and derivatives of monoclonal antibodies can be produced by techniques that are known per se in the art.

For instance, Fab or F(ab')2 fragments may be produced by protease digestion, according to conventional techniques.

Humanized antibodies can be prepared according to techniques known per se in the art. These methods involve the replacement of amino acids or domains within the sequence of the antibody, typically CDR replacement, to generate "humanized" antibodies. Methods of preparing such humanized antibodies have been disclosed for instance in Jones, Nature 321 (1986) 522).

Human antibodies may be produced according to various techniques, such as from hybridoma, or by using, for immunization, transgenic animals that have been engineered to express a human antibody repertoire (Jakobovitz et al., Nature 362 (1993) 255), or by selection of antibody repertoires using phage display methods. Such techniques are known to the skilled person and can be implemented starting from monoclonal antibodies as disclosed in the present application.

Other derivatives include chimeric antibodies as well as functionalised antibodies, i.e., antibodies that are linked to active moieties, such as a label, toxin, drug, etc.

Compositions and Administration

The invention discloses compositions that comprise an antibody as defined above, including fragments and derivatives thereof, in any suitable conditioning. The invention also provides methods of regulating NK cell functions using said antibodies and compositions. The invention also relates to methods of treating a patient, comprising administering to a patient in need thereof a amount of a composition as defined above effective for causing, facilitating, or increasing NK cell activity in said patient. The

method is more specifically directed at increasing NK cell activity in subjects having a disease caused by NK target cells, such as a cancer, infectious or immune disorder. Such methods may be used either alone or in combination with other treatments, such as radio-therapy, chemo-therapy or gene therapy, for instance.

Compositions of this invention may comprise any pharmaceutically acceptable carrier or excipient, typically buffer, isotonic solutions, aqueous suspension, optionally supplemented with stabilizing agents, preservatives, etc. Typical formulations include a saline solution and, optionally, a protecting or stabilizing molecule, such as a high molecular weight protein (e.g., human serum albumin).

Antibodies of this invention may be used to regulate NK cells in vitro, ex vivo or in vivo. For in vitro or ex vivo uses, the cells may be contacted directly with the antibody (including fragments or derivatives thereof) in any suitable device (plate, pouch, flask, etc.). For in vivo uses, the antibodies (including fragments or derivatives thereof) may be injected directly to a subject, typically by intra-venous, intra-peritoneal, intra-arterial, intra-muscular or transdermic route. Several monoclonal antibodies have been shown to be efficient in clinical situations, such as Rituxan (Rituximab) or Xolair (Omalizumab), and similar administration regimens (i.e., formulations and/or doses and/or administration protocols) may be used with the monoclonal antibodies of this invention. Furthermore, the compositions of this invention may further comprise or may be used in combination with other active agents or therapeutic programs such as chemotherapy or other immunotherapies, either simultaneously or sequentially.

Further aspects and advantages of this invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of this application.

Experimental Section

Purification of PBLs and generation of polyclonal or clonal NK cell lines.

PBLs were derived from healthy donors by Ficoll Hypaque gradients and depletion of plastic adherent cells. To obtain enriched NK cells, PBLs were incubated with anti CD3 , anti CD4 and anti HLA-DR mAbs (30mns at 4°C), followed by goat anti mouse magnetic beads (Dynal) (30 mns at 4°C) and immunomagnetic selection by methods known in the art (Pende et al., 1999). CD3 minus, CD4 minus DR minus cells are cutivated on irradiated feeder cells and 100 U/ml Interleukin 2 (Proleukin, Chiron Corporation) and 1.5 ng/ml Phytohemagglutinin A (Gibco BRL) to obtain polyclonal NK cell populations. NK cell are cloned by limiting dilution and clones of NK cells are characterized by flow cytometry for expression of cell surface receptors.

The following clones were used in this study :

CP11, CN5 and CN505 are KIR2L1 positive clones and are stained by EB6 or XA-141 antibodies.

CN12 and CP502 are KIR2DL3 positive clones and are stained by GL183 antibody.

Flow cytometry analysis

mAbs used were produced in the laboratory JT3A (IgG2a, anti CD3), EB6 and GL183 (IgG1 anti KIR2DL1 and KIR2DL3 respectively), XA-141 IgM anti KIR2DL1 (same specificity as compared to EB6, anti CD4 (HP2.6), anti DR (D1.12, IgG2a). Instead of JT3A, HP2.6, and DR1.12, commercially available mAbs of the same specificities can be used for example from Beckman coulter Inc, Fullerton, CA. EB6 and GL183 are commercially available in Beckman Coulter Inc , Fullerton, CA. XA-141 is not commercially available but EB6 can be used for control reconstitution of lysis as described in (Moretta et al., 1993).

Flow cytometry

Cells were stained with the appropriate antibodies (30mns at 4°C) followed by PE or FITC conjugated polyclonal anti mouse antibodies (Southern Biotechnology Associates Inc). Samples were analysed by cytofluorometric analysis on a FACSAN apparatus (Becton Dickinson, Mountain View, CA).

Cytotoxicity experiments

The cytolytic activity of NK clones was assessed by a standard 4hr 51Cr release assay. In which effector NK cells were tested on Cw3 or Cw4 positive cell lines known for their sensitivity to NK cell lysis. All the targets are used at 5000 cells per well in microtitration plate and the Effector on target ratio is indicated in the figures (usually 4 effectors per target cells). The cytolytic assay is performed with or without supernatant of indicated monoclonal antibodies at a ½ dilution. The procedure is essentially the same as described in (Moretta et al., 1993)

Generation of new mAbs

mAbs have been generated by immunizing 5 week old Balb C mice with activated polyclonal or monoclonal NK cell lines as described in (Moretta et al., 1990). After different cell fusions, the mAbs were fist selected for their ability to cross react with EB6 and GL183 positive NK cell lines and clones. Positive monoclonal antibodies were further screened for their ability to reconstitute lysis by EB6 positive or GL183 positive NK clones of Cw4 or Cw3 positive targets respectively.

DF200, a novel monoclonal antibody against a common determinant of KIR2D human NK receptors

One of the monoclonal antibody, the DF200 mAb, was found to react with various members of the KIR family including KIR2DL1, KIR2DL2/3. Regarding the staining of NK cells with DF200mAb both KIR2DL1+ and KIR2DL2/3+ cells were stained brightly (figure 1).

NK clones expressing one or another (or even both) of these HLAclass I-specific inhibitory receptors were used as effectors cells against target cells expressing one or more HLA-C alleles. As expected, KIR2DL1+ NK clones displayed little if any cytolytic activity against target cells expressing HLA-Cw4 and KIR2DL3+ NK clones displayed little or no activity on Cw3 postive targets. However, in the presence of DF200mAb (used to mask their KIR2DL receptors) NK clones became unable to recognize their HLA-C ligands and displayed strong cytolytic activity on Cw3 or Cw4 targets.

For example, the C1R cell line (CW4+ EBV cell line, ATCC n°CRL 1993) was not killed by KIR2DL1+ NK clones (CN5/CN505), but the inhibition could be efficiently

reverted by the use of either DF200 or a conventional anti KIR2DL1 mAb. On the other hand NK clones expressing the KIR2DL2/3+ KIR2DL1- phenotype (CN12) efficiently killed C1R and this killing was unaffected by the DF200mAb (figure 2). Similar results can be obtained with KIR2DL2- or KIR2DL3-positive NK clones on Cw3 positive targets.

Biacore analysis of DF200 mAb/ KIR 2DL1 and DF200 mAb/ KIR 2DL3 interactions.

Materials and Methods

Production and purification of recombinant proteins

The KIR 2DL1 and KIR 2DL3 recombinant proteins were produced in *E. coli*. cDNA encoding the entire extracellular domain of KIR 2DL1 and KIR 2DL3 were amplified by PCR from pCDM8 clone 47.11 vector (Biassoni et al, 1993) and RSVS(gpt)183 clone 6 vector (Wagtman et al, 1995) respectively, using the following primers:
Sense: 5'-GGAATTCCAGGAGGAATTTAAAATGCATGAGGGAGTCCACAG-3'
Anti-sense: 5'-CCCAAGCTTGGGTTATGTGACAGAAACAAGCAGTGG-3'
They were cloned into the pML1 expression vector in frame with a sequence encoding a biotinylation signal (Saulquin et al, 2003).
Protein expression was performed into the BL21(DE3) bacterial strain (Invitrogen). Transfected bacteria were grown to OD_{600}=0.6 at 37°C in medium supplemented with ampicillin (100 µg/ml) and induced with 1 mM IPTG.
Proteins were recovered from inclusion bodies under denaturing conditions (8 M urea). Refolding of the recombinant proteins was performed in Tris 20 mM, pH 7.8, NaCl 150 mM buffer containing L-arginine (400 mM, Sigma) and β-mercaptoethanol (1 mM), at RT, by decreasing the urea concentration in a six step dialysis (4, 3, 2, 1 0.5 and 0 M urea, respectively). Reduced and oxidized glutathion (5 mM and 0.5 mM respectively, Sigma) were added during the 0.5 and 0 M urea dialysis steps. Finally, the proteins were dialyzed extensively against Tris 10 mM, pH 7.5, NaCl 150 mM buffer. Soluble refolded proteins were concentrated and then purified on a Superdex 200 size-exclusion column (Pharmacia; AKTA system).

Biacore analysis.

Surface plasmon resonance measurements were performed on a Biacore apparatus (Biacore).

In all Biacore experiments HBS buffer supplemented with 0.05% surfactant P20 served as running buffer.

Protein immobilisation.

Recombinant KIR 2DL1 and KIR 2DL3 proteins were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5 (Biacore). The sensor chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimidehydrochloride and N-hydroxysuccinimide, Biacore). Proteins, in coupling buffer (10 mM acetate pH 4.5) were injected. Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH8 (Biacore).

Affinity measurements.

For kinetic measurements, various concentrations of the soluble antibody (10^{-7} to $4x10^{-10}$ M) were applied onto the immobilized sample. Measurements were performed at 20 µl/min continuous flow rate. For each cycle, the surface of the sensor chip was regenerated by 5 µl injection of 10 mM NaOH pH 11.

The BIAlogue Kinetics Evaluation program (BIAevaluation 3.1, Biacore) was used for data analysis.

Results

BIAcore analysis of DF200 mAb binding to immobilized KIR 2DL1 and KIR 2DL3.

	KD (10^{-9} M)
KIR 2DL1	**10.9 +/- 3.8**
KIR 2DL3	**2.0 +/- 1.9**

KD: Dissociation constant.

The soluble analyte (40 µl at various concentrations) was injected at a flow rate of 20 µl/min in HBS buffer, on a dextran layers containing 500 or 540 reflectance units (RU), and 1000 or 700 RU of KIR 2DL1 and KIR 2DL3 respectively. Data are representative of 6 independent experiments.

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CLAIMS

1. A monoclonal antibody, wherein said antibody binds a common determinant of KIR2D human receptors and inhibits KIR2D-mediated inhibition of NK cell cytotoxicity.

2. A monoclonal antibody of claim 1, wherein said antibody binds a common determinant of KIR2DL1, KIR2DL2 and KIR2DL3 human receptors and inhibits KIR2DL1- KIR2DL2- and KIR2DL3-mediated inhibition of NK cell cytotoxicity.

3. A monoclonal antibody of claim 2, wherein said antibody inhibits the binding of a HLA-c allele molecule having a Lys residue at position 80 to a human KIR2DL1 receptor, and the binding of a HLA-C allele molecule having an Asn residue at position 80 to human KIR2DL2 and KIR2DL3 receptors.

4. A monoclonal antibody of claim 1, wherein said antibody facilitates NK cell activity in vivo.

5. The monoclonal antibody of claim 1, wherein said antibody binds to the same epitope as monoclonal antibody DF200 produced by hybridoma DF200.

6. The monoclonal antibody of claim 1, wherein said antibody competes with monoclonal antibody DF200 produced by hybridoma DF200 for binding to a KIR receptor at the surface of a human NK cell.

7. The monoclonal antibody of claim 1, which is monoclonal antibody DF200 produced by hybridoma DF200.

8. A fragment or derivative of an antibody of claim 1, having substantially the same antigen specificity.

9. A fragment or derivative according to claim 8, which is selected from a Fab fragment, a Fab'2 fragment, a CDR and a ScFv.

10. A monoclonal antibody of claim 1, which is humanized.

11. A monoclonal antibody of claim 1, which is human.

12. A monoclonal antibody of claim 1, which is a chimeric antibody.

13. A pharmaceutical composition comprising a monoclonal antibody of claim 1 or a fragment or derivative according to claim 8, and a pharmaceutically acceptable carrier or excipient.

14. A method of regulating human NK cell activity in vitro, ex vivo or in vivo, comprising contacting human NK cells with a monoclonal antibody of claim 1 or a fragment or derivative according to claim 8.

15. A method of claim 14, for increasing the cytotoxic activity of human NK cells.

16. A method of claim 14, wherein the subject has a cancer, infectious or immune disease.

Abstract

The present invention relates to novel compositions and methods for regulating an immune response in a subject. More particularly, the invention relates to specific antibodies that regulate the activity of NK cells and allow a potentiation of NK cell cytotoxicity in mammalian subjects. The invention also relates to fragments and derivatives of such antibodies, as well as pharmaceutical compositions comprising the same and their uses, particularly in therapy, to increase NK cell activity or cytotoxicity in subjects.

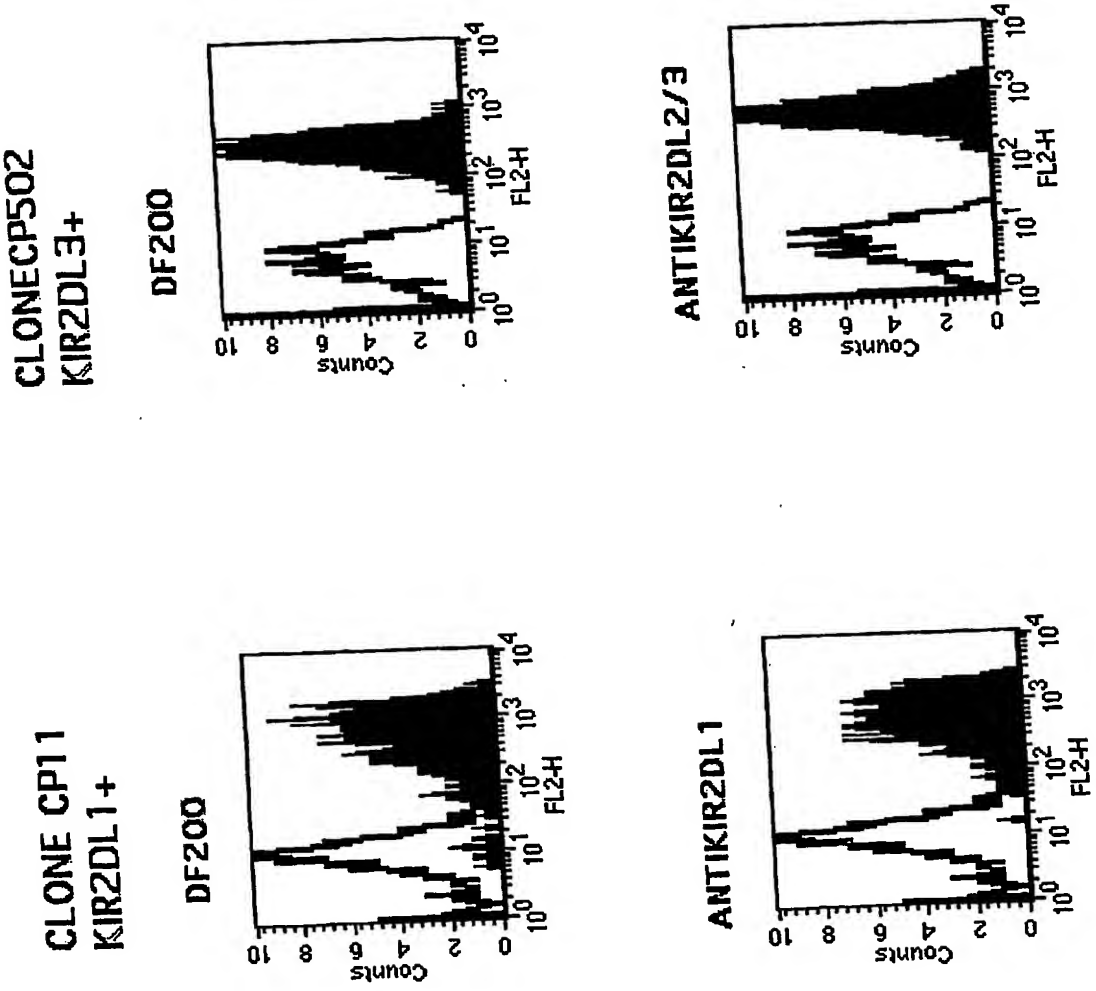
CLONECP502
KIR2DL3+
DF200
ANTIKIR2DL2/3
CLONE CP11
KIR2DL1+
DF200
ANTIKIR2DL1
Counts
FL2-H

Figure 1

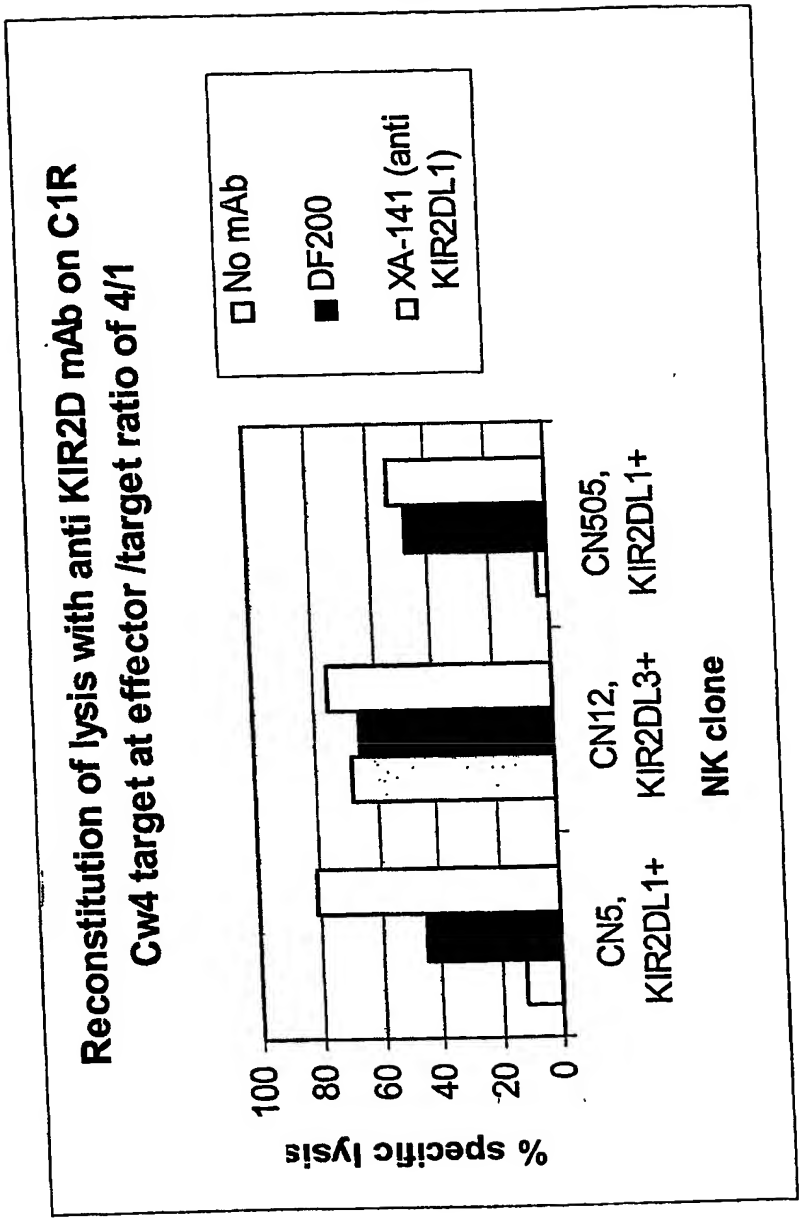
Reconstitution of lysis with anti KIR2D mAb on C1R
Cw4 target at effector /target ratio of 4/1
No mAb
DF200
XA-141 (anti KIR2DL1)
% specific lysis
100
80
60
40
20
0
CN5, KIR2DL1+
CN12, KIR2DL3+
CN505, KIR2DL1+
NK clone

Figure 2